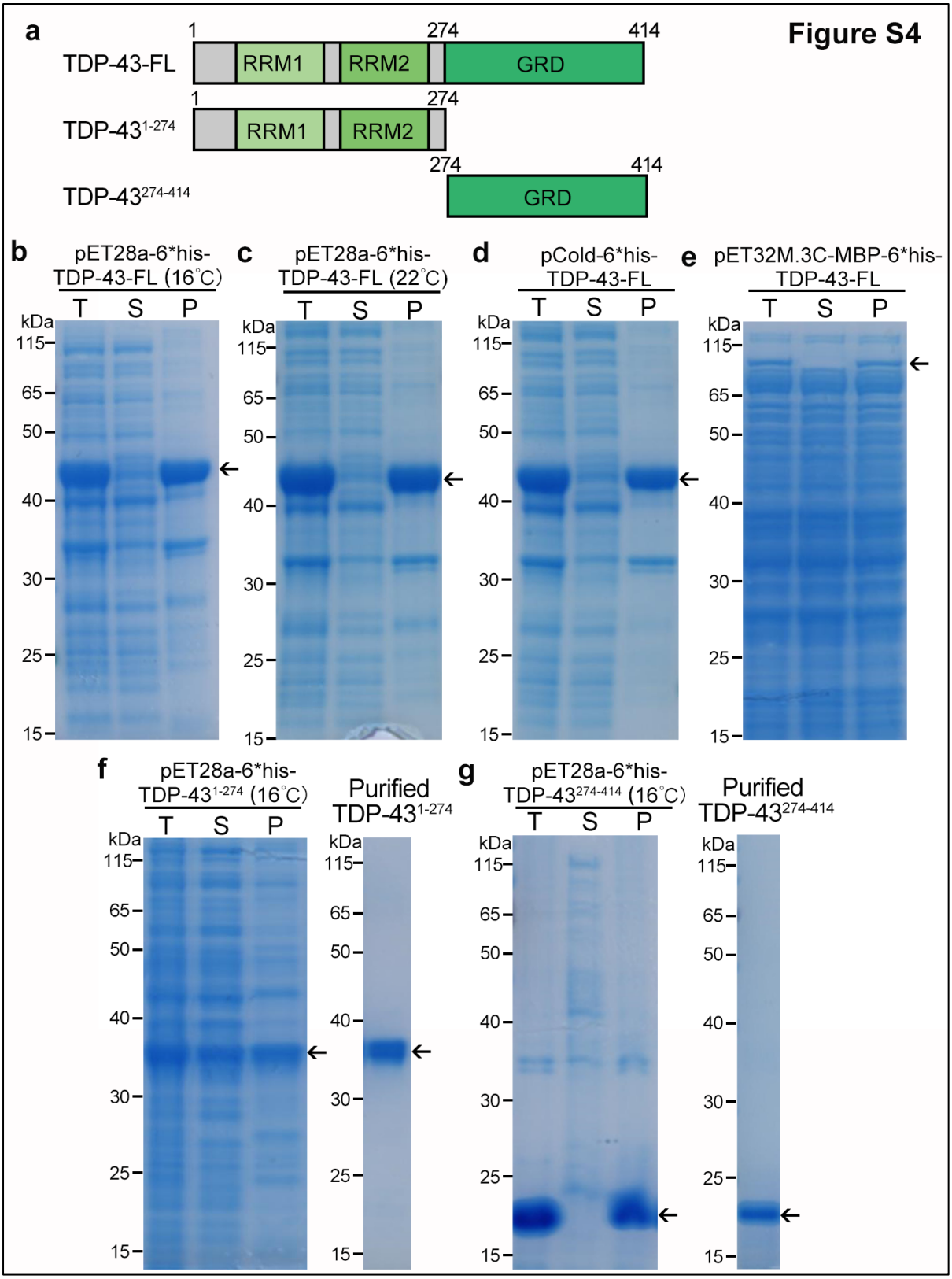
Figure S4
a
TDP-43-FL
TDP-43^1-274
TDP-43^274-414
1
274
414
RRM1
RRM2
GRD
b
pET28a-6*his-TDP-43-FL (16°C)
c
pET28a-6*his-TDP-43-FL (22°C)
d
pCold-6*his-TDP-43-FL
e
pET32M.3C-MBP-6*his-TDP-43-FL
f
pET28a-6*his-TDP-43^1-274 (16°C)
Purified TDP-43^1-274
g
pET28a-6*his-TDP-43^274-414 (16°C)
Purified TDP-43^274-414
T S P
kDa
115
65
50
40
30
25
15

Figure S4. Expression and purification of recombinant TDP-43 proteins for the *in vitro* assays. a Domains of full-length TDP-43 (TDP-43-FL), TDP-43^{1-274} and TDP-$43^{274-414}$. TDP-43-FL contains two RNA recognition motifs (RRM1/2) and a glycine-rich (GRD) domain. **b-e** Recombinant TDP-43-FL proteins expressed in the *Escherichia coli* are separated in the SDS-PAGE gel and stained with Coomassie Blue. T, total lysates; S, soluble in the lysis buffer (50 mM NaH_2PO_4, 500 mM NaCl, pH 8.0, 1 mM DTT and 1 mM PMSF); P, pellets re-suspended in 9 M of urea. TDP-43 proteins of predicated size are indicated with arrows. The solubility of TDP-43-FL protein is too low for the subsequent purification steps. This is regardless of the expression vectors used, including pET28a-6*his (**b-c**) and pCold-6*his employed by Elden et al. previously[66] (**d**), and the induction temperatures tested, such as 16 °C (**b**) and 22 °C (**c**). As an attempt to increase the solubility of TDP-43-FL, the pET32M.3C-MBP-6*his was also tried. However, it did not improve its solubility, while the total expression level is much lower than the other two vectors (**e**). **f** Given that a significant portion of the recombinant His-tagged TDP-43^{1-274} truncation protein is in the soluble fraction (left), it is subject to Ni affinity purification and HPLC (right), which is used in the *in vitro* PARylation and LLPS assays in the current study. **g** Considering that the his-tagged TDP-$43^{274-414}$ fragment is unstructured and extremely insoluble (left), it is purified using a denaturing lysis buffer containing 6 M of guanidine hydrochloride and subsequently purified using the Ni column and HPLC (right). The purified TDP-$43^{274-414}$ protein is employed in the *in vitro* assays in the present study.